

*Approved by Director: Dr. Guy Vallaro***9.1 PURPOSE:**

Only suitable and properly operating equipment and software/macros are used for casework. This protocol is to ensure that the correct parameters are monitored and equipment maintenance and performance checks are performed on critical equipment and software/macros.

9.2 RESPONSIBILITY:

DNA Section Personnel.

9.3 EQUIPMENT INVENTORY

An inventory for equipment and software/macros is maintained for the DNA section (see appendix for the software/macros). In addition, the Division of Scientific Services maintains a general laboratory inventory.

Semi-Annual: Two times within a calendar year – approximately 6 months apart.

9.3.1 Broken Equipment Notification

If a piece of equipment is not working properly, notification (visible sign) will be placed on the piece of equipment. The issue will be investigated to try to correct the problem. The equipment will remain out of use until it is deemed operational. Broken, out of service, and in need of repair equipment should be documented on its maintenance log. Notification to the Assistant Director or Deputy Director will be made.

9.4 Centrifuges

Centrifuges (used for microcon purification and concentration, see SOPs 2 and 20) will be annually performance checked on site by a qualified vendor. Records will be kept by the Quality Section. If a centrifuge needs repair, it will be put out of use and sent out for repair. Quarterly cleaning of centrifuges will be documented on DNA QR-290.

9.5 Thermometer Calibration Verification:

Performance Check each thermometer against a NIST traceable thermometer prior to its first use and annually thereafter.

The actual NIST traceable thermometers used for critical temperature checks (i.e. 56°, 70° used for extraction incubations) will also be performance checked annually by placing the thermometers in boiling water, at room temperature, ice and/or dry ice as necessary by the thermometer's reading capabilities. NIST Traceable thermometers are considered valid for 5 years.

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9.5.1 The annual performance check of thermometers will be done following the directions below: Each thermometer will be checked against a NIST traceable thermometer prior to its first use and then annually. Calibration may be checked against a certified purchased thermometer by placing the certified thermometer and the thermometer being checked in the freezer, refrigerator, heat block or incubator, leaving them in for at least one hour, and recording the temperature of both thermometers. An acceptable difference between readings from the two thermometers is $\pm 2^{\circ}\text{C}$. If the thermometer being calibrated does not read $\pm 2^{\circ}\text{C}$ of the traceable thermometer, continue to monitor for another day. If required, discard it in the proper manner and replace it with an acceptable one.

9.5.2 Following the verification of calibration, mark the top of the thermometer to indicate that it is properly calibrated.

9.6 Refrigerators/Freezers:

9.6.1 An assigned calibrated thermometer is inside each refrigerator/freezer.

9.6.2 Log the temperature weekly on the appropriate sheet (DNA QR-200a/b).

9.6.3 If the temperature in the unit is more than $\pm 5^{\circ}\text{C}$ (freezer) or $\pm 3^{\circ}\text{C}$ (refrigerator) above the set temperature, perform appropriate maintenance measures. Such measures would include readjusting the temperature settings, defrosting the unit, or having the unit serviced.

9.6.4 Record any maintenance or adjustment to each unit on the appropriate log sheet. Please use DNA QR-200a and 200b.

9.7 Pipettes:

All pipettes located in the DNA Unit will be performance checked annually by a qualified vendor. Repair and calibrations will be noted on certificate from vendor if performed. Pipettes used for DNA amplification will be annually performance checked and calibrated or repaired by an ISO 17025 calibration vendor. Records will be kept by the Quality Section.

9.7.1 Pipettes are individually identified and tracked (pipette log) either by serial number or numeric designation and are generally assigned to a specific workspace.

9.7.2 If a pipette in the DNA Unit is found "out of tolerance" during the annual performance check, and is found to be greater than 20% out of tolerance, a QAR will be opened to investigate this incident.

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- 9.8.1 All ovens have assigned thermometers.
- 9.8.2 Turn the oven on (if necessary). Set the oven to the desired temperature. After ~20 minutes, check the thermometer.
- 9.8.3 If the actual temperature of an oven has deviated from the set temperature by more than $\pm 1^{\circ}\text{C}$ the temperature is readjusted.
- 9.8.4 Each time the oven is used, any maintenance and adjustments are logged on to the appropriate sheet. (See DNA QR-204)

9.9 Mettler Balance:

- 9.9.1 Before each use, verify that the unit is level and clean.
- 9.9.2 The Mettler must be calibrated once a year by an authorized service representative; Service dates are on the label on the balance.

9.10 Denver Instrument UltraBasic pH Meter:

- 9.10.1 The pH meter is standardized prior to each use. Prior to each new standardization, previous standardizations will be cleared.
- 9.10.2 Please refer to DNA WI-29 (pH Meter) for further instruction.
- 9.10.3 Each calibration is logged onto the proper log sheet (Please use DNA QR 206a and 206b or QRM 48).
- 9.10.4 If the instrument does not calibrate properly, follow the troubleshooting guidelines found in the Operational Manual. If problems persist the instrument will be returned to the manufacturer for service.
- 9.10.5 Electrode is stored in a 3M KCL solution. The KCL can be in-house formulated or commercially purchased. Please refer to WI-29 (pH Meter) and QR-267 (3M KCL).

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- 9.11.1 Log each use of a thermal cycler on the appropriate case amplification worksheet.
- 9.11.2 Each Thermal Cycler will be performance checked annually by a qualified outside vendor using a NIST traceable temperature measuring device. Certificates will be maintained for each thermal cycler by the Quality Section.
- 9.11.3 If Thermal Cycler needs repair or further adjustment, it will be quarantined and sent out for repair. Upon return, a performance check will be done before the instrument is reinstated for casework. (Please use DNA QR-259)
- 9.11.4 Each Thermal Cycler's block (including wells) will be cleaned approximately every three months. This is documented on DNA QR-288.

9.12 ABI 3130xl Genetic Analyzer:

- 9.12.1 The ABI 3130xl Genetic Analyzers are serviced annually by factory service contract. The ABI service department is contacted for repairs and the annual service. A performance check will be done following repair or annual service and before the instrument is reinstated for casework. The performance check will include the methodologies that represent the different types of dye sets used for casework. (Document Performance Check by using DNA QR-260)
- 9.12.2 During periods of operation, additional maintenance is performed on the ABI 3130 Genetic Analyzers and is documented on the appropriate quality record (QR-222) and kept in a maintenance binder by the DNA section. Buffer and water are changed as required and the septa are rinsed after each buffer and water change. As required, the polymer is changed, the water wash wizard is run, the water trap is flushed, and the computer is restarted. Please refer to DNA SOP-4 for more detailed information on maintenance and cleaning.

9.13 ABI 7500 Real-Time Instrument Maintenance:

- 9.13.1 The ABI 7500 Real-Time instruments are serviced annually by factory service contract. The ABI service department is contacted for repairs and the annual service. Records are maintained in 7500 Performance Check Notebook.

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- 9.13.2 Annually: Instrument performance is verified following the annual service using an ABI TaqMan RNase P verification plate per manufacturer's protocols and documented on QR-262. Records are maintained in Performance Check Notebook.
- 9.13.3 During periods of operation, additional maintenance is performed on the ABI 7500 instruments by the Division of Scientific Services and is documented on the appropriate log sheet (DNA QR-18):
- 9.13.4 Approximately every 2 months: background check and block cleaning as necessary (DNA QR-18).
- 9.13.5 Semi-annually (twice a year): spectral calibrations (Pure Dye) per manufacturer's protocols. (One is performed in association with the annual service). Documented on DNA QR-18.
- 9.13.6 A performance check will be done before the instrument is reinstated for casework as follows:
- Following the annual preventative maintenance (performed by an ABI service engineer), the performance check is as described in 9.13.2 and is documented using DNA QR-262.
- Following an instrument repair (performed by an ABI service engineer at any other time that year) or following the spectral calibrations (Pure Dye) (performed by laboratory personnel at the 6-month interval following the annual PM), the performance check is done by running a "Quality Controlled Passed" set of DNA standards and a no template control and documented using DNA QR-34 for Quantifiler Trio.
- 9.13.7 Please refer to ABI 7500 User's Manual for instructions for performing maintenance procedures.

9.14 Biomek 3000:

On-site vendor calibration/Preventative Maintenance occurs once a year. A performance check is completed before the instrument is used for casework. Please use DNA QR-286 and QR-287. See DNA SOP-28 for further guidance. If the instrument is moved or the deck is completely disassembled (i.e. for cleaning, maintenance, etc.), the deck (base, left, and right modules) is realigned and the instrument is performance checked.

9.15 BSD Duet:

Calibration/Preventative Maintenance occurs twice a year. This may be performed by an analyst or

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vendor. A performance check is completed after the instrument has been serviced or moved and before the instrument is used for casework. See DNA SOP-28 and use DNA QR-265 for performance checking and DNA QR-296 for maintenance.

9.16 EZ1 Advanced XL:

The Qiagen EZ1 Advanced XL Extraction Instrument is serviced annually by factory service contract. The service department is contacted for repairs and the annual service. A performance check is completed after the instrument has been serviced and before its use for casework. For documentation of a performance check use DNA-QR-277. Records are maintained in the EZ1 Performance Check/Maintenance/Service Notebook.

9.16.1 If any of the above instruments or equipment have any issues with performance checks or maintenance, notification to the TL and Assistant Director or Deputy Director will be made.

9.17 Software/Macros: See the Appendix below for details

9.17.1 Analysis Software/Statistic Macros Performance Check:

GeneMarker HID, GeneMapper, STRmix, DNA-QR-46 Probability of Kinship Worksheet, DNA QR-43 US Y-STR DB Searcher, and DNA QR-44 Match Probability of Parentage Worksheet are software/macros used in the DNA Unit for analysis and statistics. A set of data with the expected results for each of the above software/macros will be kept on the U: Drive and used to conduct the performance check on the above softwares/macros semi-annually (approximately every 6 months). See Appendix below for corresponding Quality Records for the various performance checks. Further guidance and details can be found on these DNA QRs. QRs can be found in Qualtrax or if they are macro workbooks, on the Shared drive in the Controlled Document Folder under DNA.

9.17.1.1 STRmix Performance Check

For STRmix, performance checks will be run with both the same seed and different seeds using samples from F6C and IDP. If there is an upgrade or software patch for STRmix, the performance check will follow the recommendation of the manufacturer. The results will be documented on DNA QR-306. Further guidance/direction is located in the DNA QR-306 workbook.

9.17.2 Processing Macros Performance Check:

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For Macros used throughout the case flow process, a Performance Check will be performed semi-annually. A DNA analyst (other than the macro's creator) will conduct the performance check during his/her proficiency testing from the beginning of the DNA extraction to issuing the DNA report (if applicable). The results will be documented on DNA QR-292 (nuclear) DNA QR-293 (mtDNA). Further guidance and details can be found on these DNA QRs. See Appendix below for corresponding macros that will be performance checked on DNA QR-292. DNA QRs can be found in Qualtrax or if they are macro workbooks; on the Shared drive in the Controlled Document Folder under DNA.

For the STRmix Known Input File Generator (DNA-QR-304), a performance check will be done semi-annually by an analyst in the Database Unit. Please refer to DNA QR-282 for details.

9.17.3 Software/Macros Updates:

After a software/macro update, upgrade or software patch and prior to casework commencing, a performance check will be conducted. For software, performance checks will be conducted by any DNA analysts. For excel macros, performance checks will be conducted by any DNA analyst other than the macro's creator. The results of the performance check will be documented on the appropriate QR (see appendix) and notes will describe reasons the performance check was performed.

9.17.4 Out of Service Software/Macros Notification:

If a software/macro is not working properly, notification will be sent out to the DNA Unit through email by the Assistant Director, or designee. The issue, such as a bug in the macro code, will be investigated to try to correct the problem. The software/macro will remain out of use until it is deemed operational. Appropriate performance check will be conducted by the DNA analysts. For macros, performance checks will be conducted by the DNA analysts and the macro's creator. Documentation will be made on the appropriate QR sheet. After the issue has been remedied, the Assistant Director, or designee will notify the DNA Unit that the software/macro is fit for use in casework.

9.17.5 CODIS

There will be a quarterly performance check of the CODIS software following the directions on DNA QR-291 (in Qualtrax) and the results will be documented on DNA QR-291. See DNA QR-291 for further details and guidance.

A semi-annual check of the CODIS search parameters will also be performed. An email will be sent by the CODIS administrator or alternate after the completion of this check. The Quality Manager maintains a copy of this email.

DNA SOP-9 Equipment & Performance Checks

Document ID: 929

Revision: 6

Effective Date: 8/30/2017

Status: Published

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*Approved by Director: Dr. Guy Vallaro***Appendix****Analysis and Statistic Software/Macros**

QR Name	Description	QR	Location of QR
GeneMapper	ID/IDP and Yfiler data analysis	DNA QR-294	Qualtrax
GeneMarker	Fusion 6C data analysis	DNA QR-294	Qualtrax
STRmix	Deconvolution and HPD calculation	DNA QR-306	S Drive
DNA QR-44	Match Probability of Parentage Worksheet	DNA QR-280	Qualtrax
DNA QR-43 US Y-STR DB Searcher	Y-Stats Workbook	DNA QR-280	Qualtrax
DNA-QR-46 Probability of Kinship Worksheet	Kinship Probability workbook	DNA QR-278	Qualtrax

Casework Processing Macros

QR Name	Description	QR	Location of QR
DNA-QR-25,26,27 EZ1 Extraction Worksheets	EZ1 Worksheet - Knowns	DNA QR-292	Qualtrax
DNA-QR-25,26,27 EZ1 Extraction Worksheets	EZ1 Worksheet - NonDifferentials	DNA QR-292	Qualtrax
DNA-QR-25,26,27 EZ1 Extraction Worksheets	EZ1 Worksheet - Differentials	DNA QR-292	Qualtrax
DNA-QR-24 Quantifiler Trio Worksheet	Quantifiler Trio Worksheet	DNA QR-292	Qualtrax
DNA-QR-22b,32 Dilution Worksheet and Quant Trio Report - F6C	Dilution Worksheet - F6C	DNA QR-292	Qualtrax
DNA-QR-3,299 Amplification Worksheets	YF Amplification Worksheet	DNA QR-292	Qualtrax
DNA-QR-3,299 Amplification Worksheets	F6C Amplification Worksheet	DNA QR-292	Qualtrax
DNA-QR-16e,300 Injection Worksheets	F6C Injection Worksheet	DNA QR-292	Qualtrax
DNA-QR-16e,300 Injection Worksheets	YF Injection Worksheet	DNA QR-292	Qualtrax
DNA-QR-20 Staff Search Worksheet	Staff Search	DNA QR-292	Qualtrax
Cross-Comparison & Staff Search Tool	Cross-Comparison/Staff Search	DNA QR-292	Qualtrax

**State of Connecticut Department of Emergency Services and Public Protection
Division of Scientific Services**

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DNA-QR-37 GeneMarker Concordance Checker	GeneMarker Concordance Checker	DNA QR-292	Qualtrax
DNA-QR-45E GeneMapper Concordance Checker	GMID Concordance Checker - YF	DNA QR-292	Qualtrax
QR Name	Description	QR	Location of QR
DNA-QR-301 Project Comparison Tool	Project Comparison Tool	DNA QR-292	Qualtrax
DNA-QR-302 Contributor Estimation Worksheet	Contributor Estimator	DNA QR-292	Qualtrax
DNA-QR-303 STRmix Secondary Diagnostic Output Review for Fusion 6C Data	STRmix Secondary Diagnostics	DNA QR-292	Qualtrax
mtDNA Workbook	mtDNA Workbook	DNA QR-293	Qualtrax
DNA-QR-304 STRmix Known Input File Generator	CO to STRmix Converter/Input file generator	DNA QR-282	Qualtrax